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Development of Nerve Cells in Hydrozoan Planulae: I. Differentiation of Ganglionic Cells

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Abstract. The cytomorphosis of ganglionic cells in hydrozoan planulae of *Halocordyle disticha* is described at the fine-structural level. Ganglionic cells arise from undifferentiated interstitial cells (I-cells). I-cells originate at 8 hours postfertilization as a central core of cells in the endoderm. Such I-cells possess a centrally located nucleus with one to several tiny nucleoli, a few segments of rough endoplasmic reticulum, a few mitochondria, electron-dense granules, and free ribosomes. Interstitial cells destined to form ganglionic cells migrate as single cells through the mesoglea to the base of the ectoderm where they can divide and subsequently differentiate. Early stages of neural differentiation are characterized by the enlargement of the I-cell nucleoli, the loss of electron-dense granules, and the appearance of a Golgi complex, numerous mitochondria, and microtubules. Next, neurites grow out from both sides of the ganglionic cell body and join neurites from adjacent ganglionic cells to form an extensive neural plexus just apical to the mesoglea in the planula. Neurites are rich in mitochondria and microtubules and extend in both a longitudinal and transverse direction with respect to the planular anterior-posterior axis. Some neurites extend down from the neural plexus and abut the mesoglea. The last phase of ganglionic cell development occurs when electron-dense droplets appear within the region of the Golgi and eventually in the neurites. Neurites contain both electron-dense droplets and dense cored vesicles located in clusters at specific intervals along their length. Such droplets and vesicles are often found in close association with the mesoglea in neurites which contact the mesoglea. Two morphological types of ganglionic cells are identifiable at the fine-structural level: a light ganglionic cell and a dark ganglionic cell. Light ganglionic cells possess an electron-lucent cytoplasm and fewer mitochondria than dark ganglionic cells. Dark ganglionic cells have an electron-dense cytoplasm and their cell bodies are more oblong than the rounder cell bodies of light ganglionic cells. Light ganglionic cells comprise the majority of the planular ganglionic population.

Neural differentiation begins in the planular ectoderm at 24 hours postfertilization and continues throughout larval development. The neural plexus is present at 24 hours and increases in size (*i.e.*, number of neurites) and complexity as animals mature. Ganglionic cells are distributed along the entire length of the planula and steadily increase in number as planulae age. All stages of ganglionic cell differentiation are found throughout planular development (24–96 hours postfertilization). Ganglionic cells of the planula show combined morphological features of interneurons and neurosecretory cells, suggesting they are multifunctional neurons.

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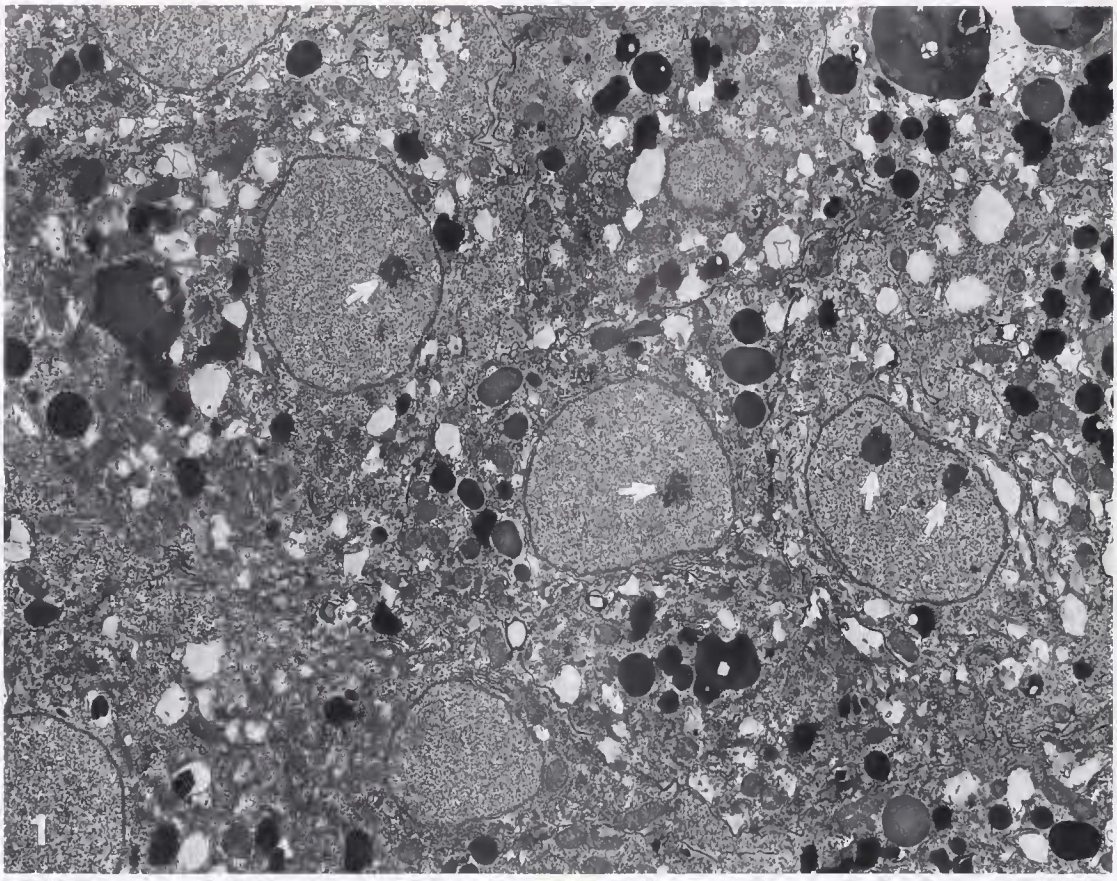
Introduction

Cnidarians possess the most primitive nervous systems of all metazoans. Examination of the neurobiology of these animals is important because studying such lower phyla may help us understand: 1) the principles on which nervous systems work; 2) important neural similarities and differences among animal groups; and 3) the genesis and early evolution of the nervous system.

Hydrozoan planulae provide excellent developmental systems in which to examine patterns of neural differentiation (Martin and Archer, 1986). Ultrastructural examination of the nerve elements in mature planulae of four species of hydrozoans, *Halocordyle disticha* (*Pennaria tiarella*) (Martin and Thomas, 1980), *Mitrocomella polydiademata* (Martin *et al.*, 1983), *Hydractinia echinata* (Weis *et al.*, 1985), and *Phialidium gregarium* (Thomas *et al.*, 1987) indicates that the hydrozoan planular nervous system is composed of at least two cell types, sensory cells and ganglionic cells. Sensory cells extend from the free surface of the planula to the mesoglea and are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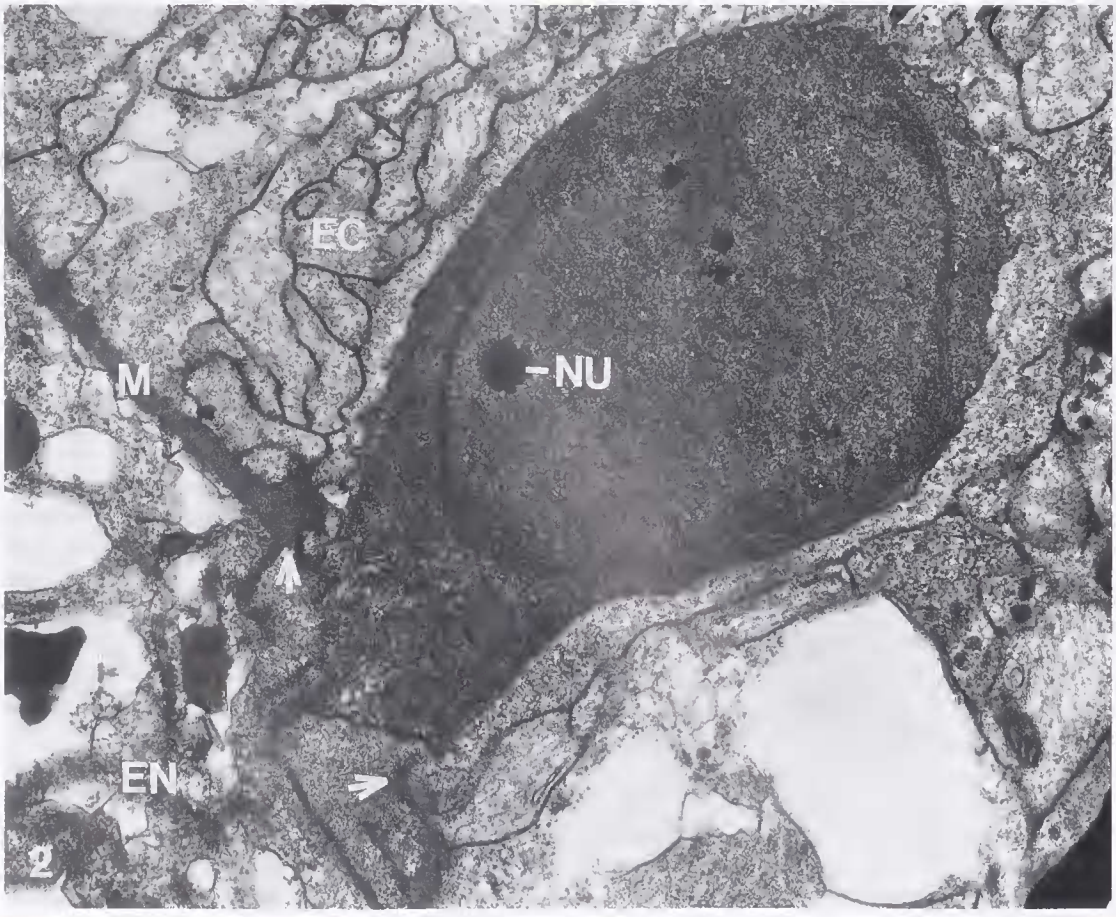
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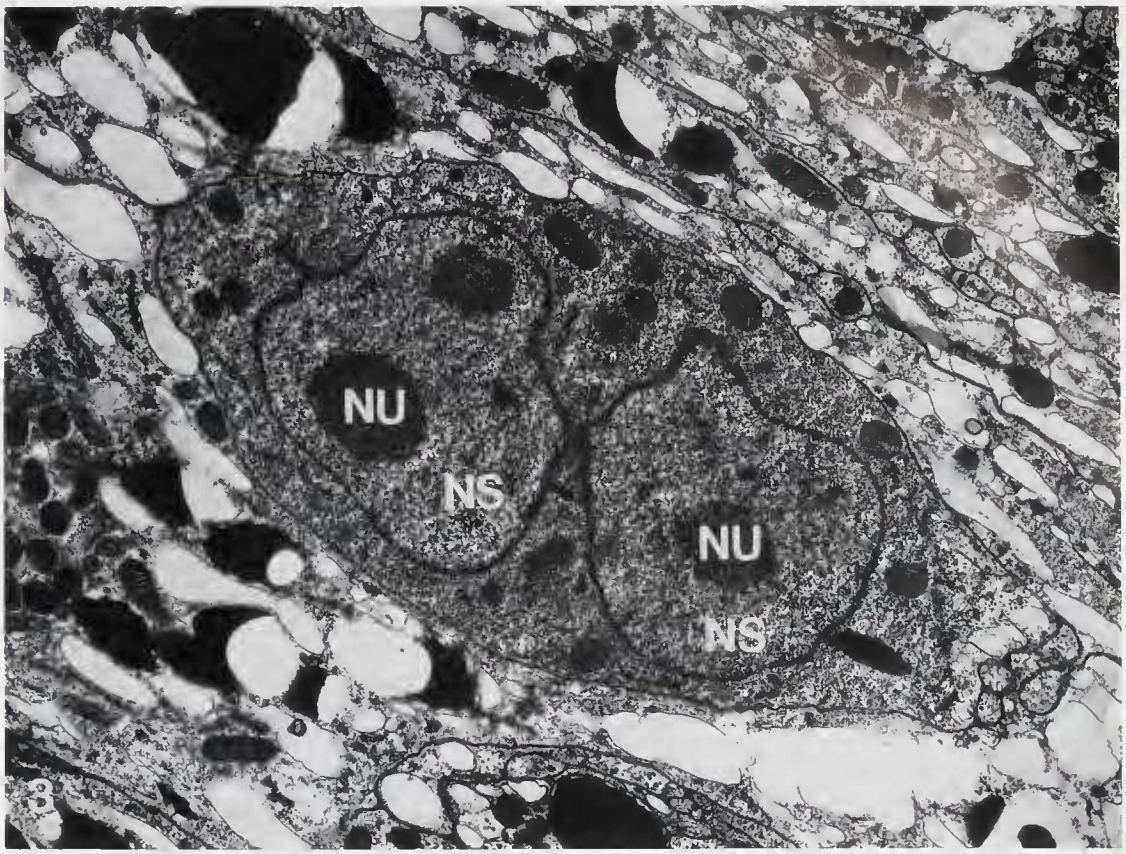


Figure 3. Dividing "neural" interstitial cell at the base of the ectodermal epithelium. Once such divisions are completed the progeny begin to differentiate into ganglionic cells. Such ganglionic cells grow neurites in between the cell bodies causing the cell bodies of the progeny to move apart. NS, nucleus; NU, nucleolus. ×11,670.

characterized by a single apical cilium and numerous cytoplasmic microtubules and neurosecretory droplets. Martin and Thomas (1980) and Thomas *et al.* (1987) demonstrated that planular sensory cells are derivatives of the ectodermal epithelium. Ganglionic cells are found at the base of the ectoderm and possess neurites rich in microtubules and mitochondria. Ganglionic cells arise from undifferentiated interstitial cells (Martin and Thomas, 1981a, b). Surprisingly, the cytomorphosis of sensory cells (Martin, submitted) and ganglionic cells in planulae has never been described.

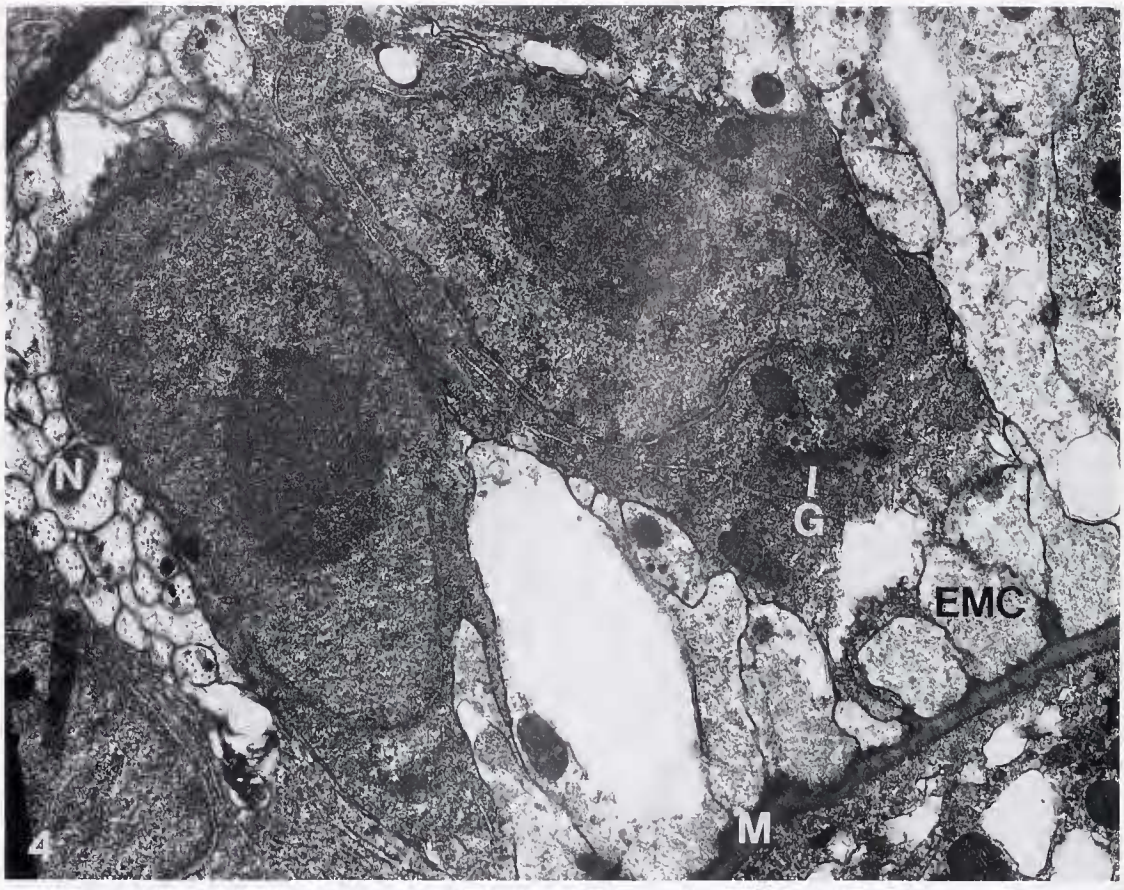
This study employs transmission electron microscopy to examine the differentiation of ganglionic cells in hydrozoan planulae of *Halocordyle disticha.* Beginning with the young interstitial cell and ending with the fully formed ganglionic cell and its neurites, this work presents the first comprehensive morphological description of ganglionic cell differentiation and genesis of the neural plexus in the planula, identifies two morphological types of ganglionic cells in the planula, and provides morphological evidence suggesting that larval ganglionic cells are multifunctional neurons.

Materials and Methods

Colonies of the marine hydrozoan *Halocordyle disticha* were collected from pier pilings in Morehead City, North Carolina. Fronds from male and female colonies

Figure 1. Interstitial cells in the endoderm of an 8 hour embryo. These cells are undifferentiated cells that contain a centrally located nucleus with one to several nucleoli (arrows). A few segments of rough endoplasmic reticulum, a few mitochondria and electron-dense granules, and ribosomes occupy the cytoplasm of the cell. ×5700.

Figure 2. Migrating interstitial cell in the mesoglea region. The cell has a trailing pseudopod and the mesoglea is disrupted (arrows) in the region of cell contact. Examination of serial sections confirms the broken nature of the mesoglea. EC, ectoderm; EN, endoderm; M, mesoglea; NU, nucleolus. ×21,000.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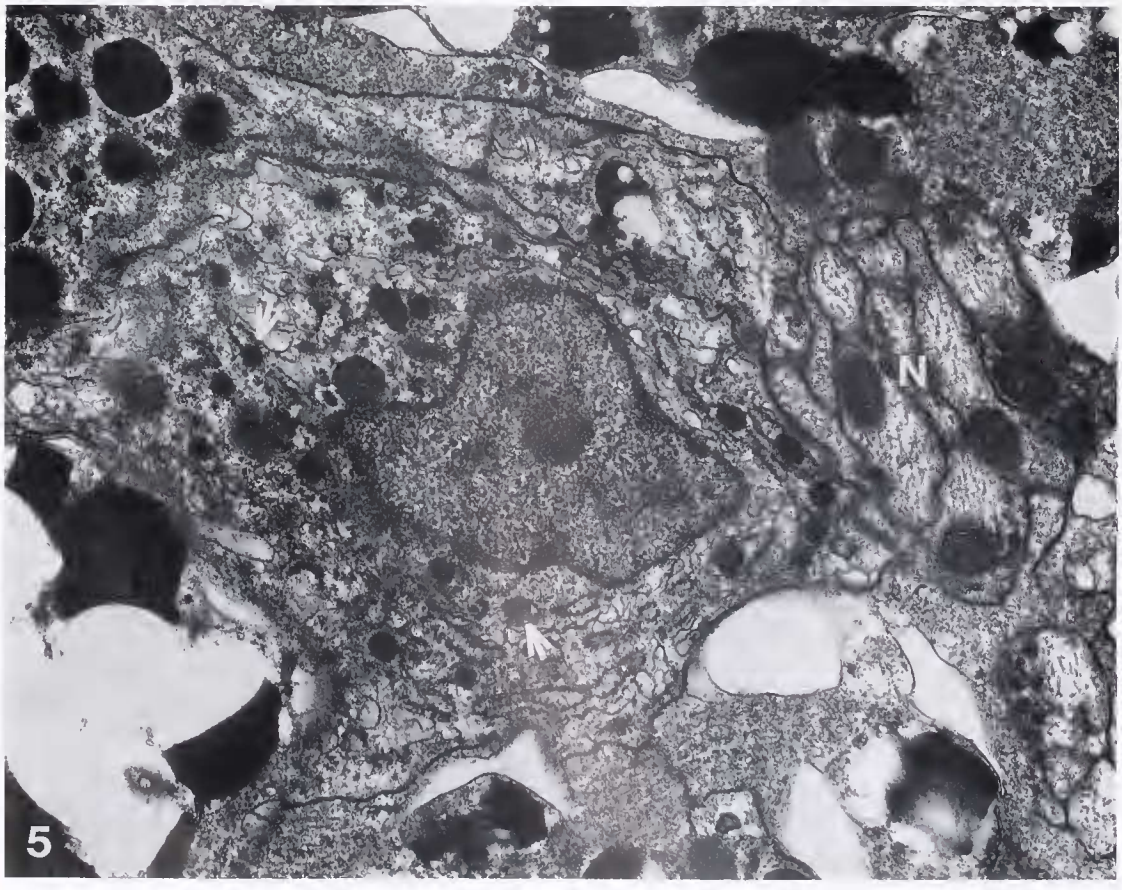
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were placed together in large finger bowls of filtered seawater. Bowls were placed in the dark at 6:00 pm and returned to the light at 9:00 pm. Shortly after exposure to light, early cleavage embryos appeared in the bottoms of the dishes. Embryos were collected, placed in small finger bowls of filtered seawater, and reared at 23°C.

Eight-hour embryos and 16-, 24-, 48-, 72-, and 96-hour planulae were processed for transmission electron microscopy. Samples were fixed for 1 hour in 2.5% glutaraldehyde, pH 7.4, in 0.2 *M* phosphate buffer. They were postfixed for 1 hour in 2% osmium tetroxide, pH 7.2, in 1.25% sodium bicarbonate. Specimens were dehydrated in an ethanol series, infiltrated, and embedded in Spurr's embedding media. Blocks were serially thin-sectioned on a Porter-Blum MT-2B ultramicrotome, placed on 150-mesh copper grids, and stained with 3.5% uranyl acetate in ethanol followed by lead hydroxide. Grids were examined and photographed with a Hitachi H-600 transmission electron microscope.

Results

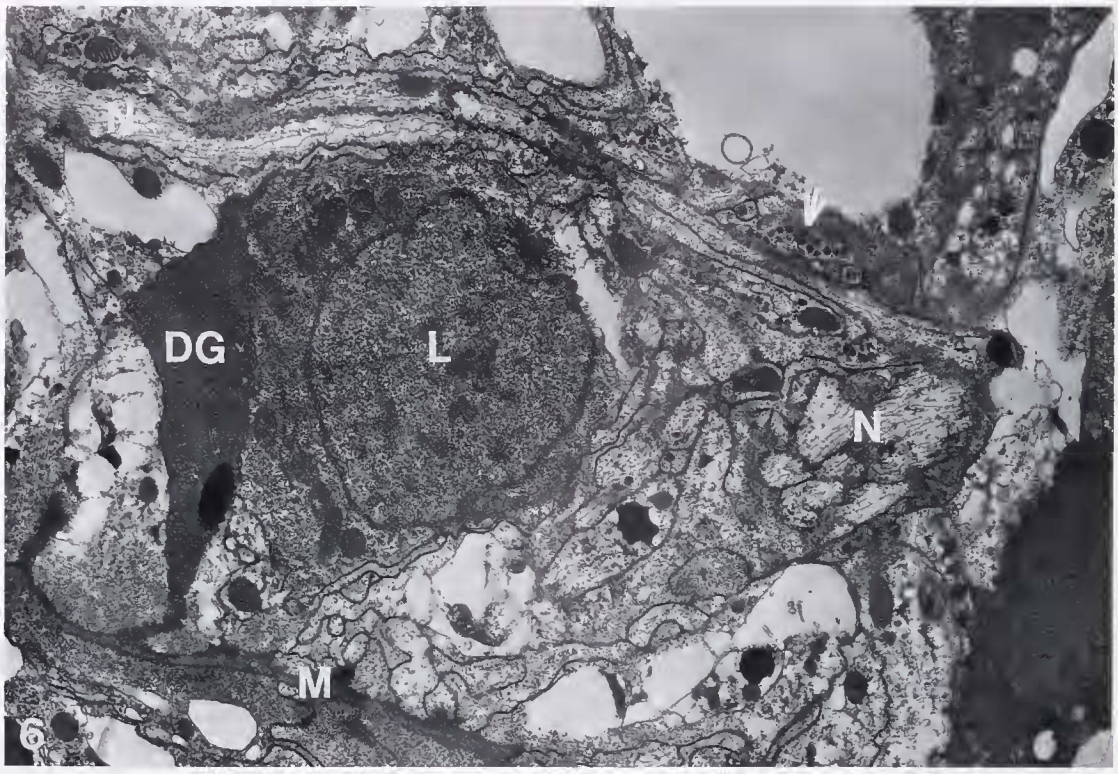
In *Halocordyle,* ganglionic cells form from undifferentiated interstitial cells (Martin and Thomas, 1981a, b). The interstitial cells arise during gastrulation (8–10 hours postfertilization) as a central core of cells located in the endoderm (Fig. 1). These young interstitial cells are round, measuring *ca.* 7 μm in diameter. These early I-cells contain a centrally located nucleus with one to several tiny nucleoli. Free ribosomes, a few mitochondria, and a few segments of rough endoplasmic reticulum may fill the cytoplasm of the I-cell. Small electron-dense granules may also be present in the cytoplasm. As interstitial cells mature, these electron-dense granules are lost and the nucleoli greatly enlarge.

Interstitial cells destined to form ganglionic cells emigrate as single cells from the endoderm to the ectoderm from all positions along the anterior-posterior axis of the planula. (Fig. 2). Such migrating I-cells possess a trailing pseudopod. As I-cells cross the mesoglea, the mesoglea appears to break in the region of transection (Fig. 2). Once I-cells have moved into the ectoderm the broken ends of mesoglea appear to reconnect. I-cells migrate to the base of the ectoderm, stop, sometimes divide, and subsequently develop morphological characteristics of ganglionic cells (Figs. 3–9). I-cells continually migrate to the base of the ectoderm throughout larval development. Cell bodies of fully differentiated ganglionic cells measure *ca.* 5 μm in diameter and are first detected in the ectoderm at 24 hours postfertilization. Ganglionic cells are distributed along the entire anterior-posterior axis of the planula. They increase in number as development proceeds and are found in the planula throughout its remaining larval life.

Once "neural" interstitial cells have migrated to the ectoderm they locate in the interstitial spaces just apical to the foot processes of the epitheliomuscle cells. These "neural" I-cells may undergo division in the ectoderm (Fig. 3) and then differentiate into ganglionic cells (Figs. 4–9). The nucleoli of the forming ganglionic cells enlarge and the nuclei exhibit condensed chromatin (Figs. 4–7). If multiple nucleoli are present in the nuclei of these forming ganglionic cells, they appear to eventually coalesce to form a single large nucleolus. A Golgi complex, segments of rough endoplasmic reticulum, mitochondria, and microtubules appear in the cytoplasm of the differentiating cell (Figs. 4–7). Shortly after these organelles appear, neurites begin to form (Figs. 4, 5). Such neurites grow out from both sides of the cell body and are rich in microtubules and mitochondria (Fig. 5). These growing neurites make contact with neurites from adjacent ganglionic cells thus forming a neural plexus at the base of the ectoderm. As the neurites are forming, electron-dense droplets appear within the rough endoplasmic reticulum and the Golgi region of the perikaryon (Fig. 5). Shortly after these droplets appear in the cell body region they also are seen in the neurites (Figs. 5, 6). These droplets appear not to be packaged in membranes. Membrane-bound dense cored vesicles are visible in the

Figure 4. Developing ganglionic cells at the base of the ectodermal epithelium of a 24 hour planula. Interstitial cells destined to form ganglionic cells migrate to the ectoderm and locate in the interstitial spaces just apical to the foot processes of the epitheliomuscle cells (EMC). Once in position, these I-cells insert themselves into a forming neurite plexus and develop morphological characteristics of ganglionic cells. Presumably the profiles of neurites (N) that are visible in this micrograph have arisen from interstitial cells which previously differentiated. Early stages of ganglionic cell differentiation include the appearance of a Golgi complex (G), a few segments of rough endoplasmic reticulum, and a few mitochondria in the cell cytoplasm. M, mesoglea. ×14,700.

Figure 5. Differentiating ganglionic cell in a 24 hour planula. The cytoplasm becomes filled with mitochondria and microtubules. Neurites (N) begin to grow out from the cell body in both a transverse and longitudinal direction. The last phase of neural differentiation is characterized by the appearance of electron-dense droplets (arrows) in the cytoplasm of the cell body and in the neurites (N). These droplets first appear in the region of the rough endoplasmic reticulum and then in the region of the Golgi. Droplets in the Golgi are smaller in diameter than in the rough endoplasmic reticulum suggesting that the material is compacted as it moves through the cytoplasm of the perikaryon. ×18,700.



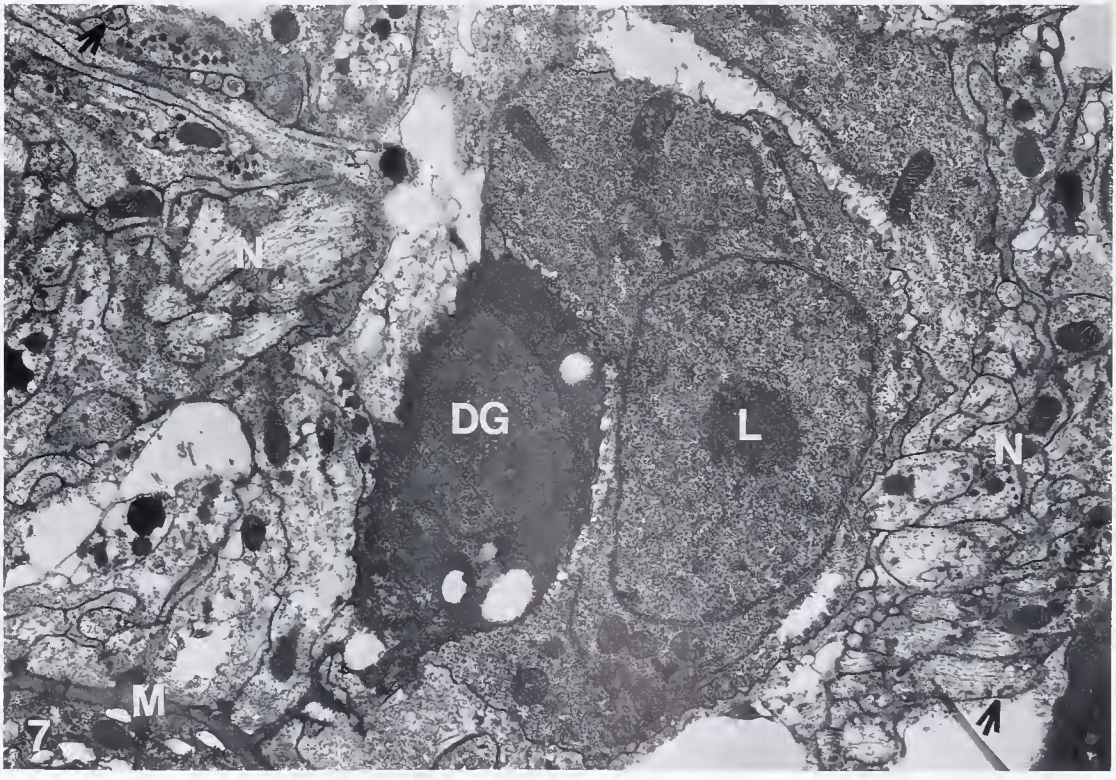
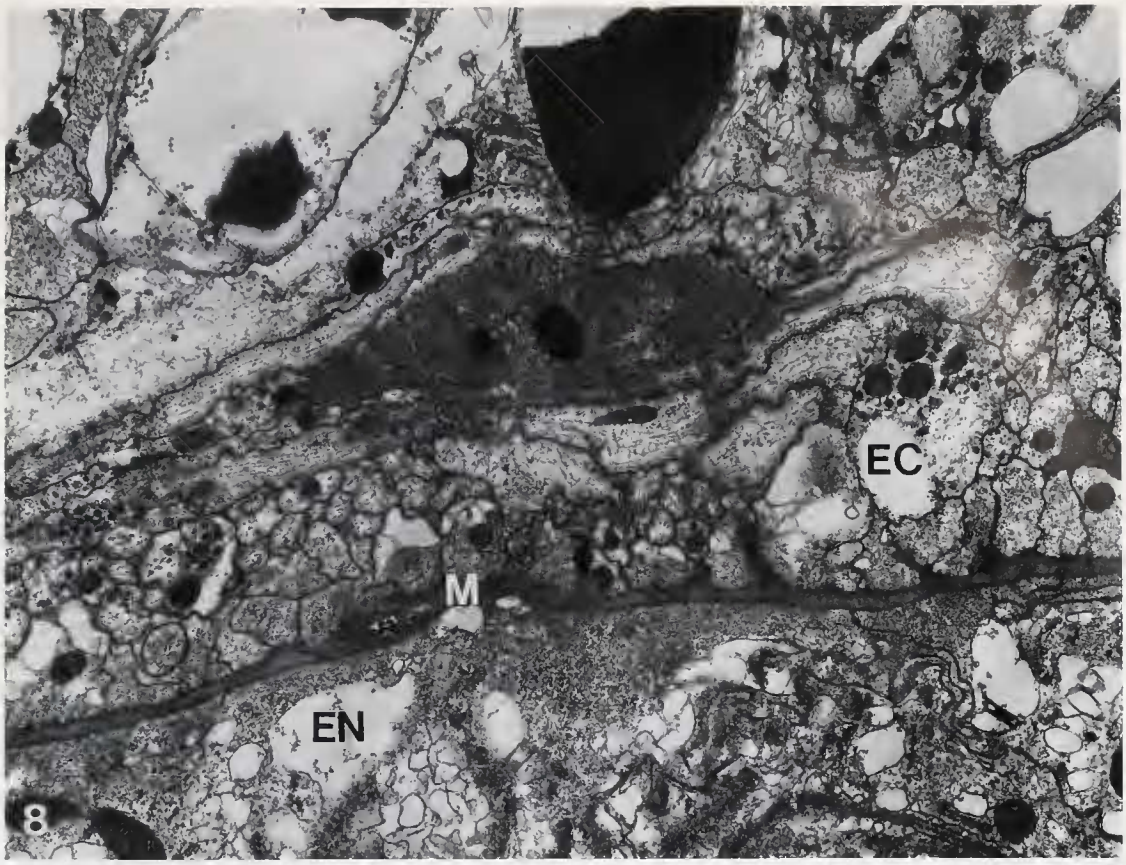


Figure 6. Dark and light ganglionic cells in a 24 hour planula. Light ganglionic cells (L) comprise the majority of the ganglionic cell population. They have an electron-lucent cytoplasm rich in microtubules and mitochondria. A portion of a dark ganglionic cell (DG) is visible in close association with the light ganglionic cell. Both cells contribute neurites (N) rich in microtubules, mitochondria, and electron-dense droplets (arrow) to the plexus. M, mesoglea. ×10,000.

Figure 7. Dark and light ganglionic cells at the base of the ectoderm of a 24 hour planula. Dark ganglionic cells (DG) are smaller than light ganglionic cells (L) and possess an abundance of mitochondria. Neurites (N) extend from both cells. Arrows, dense cored vesicles; M, mesoglea. ×10,000.



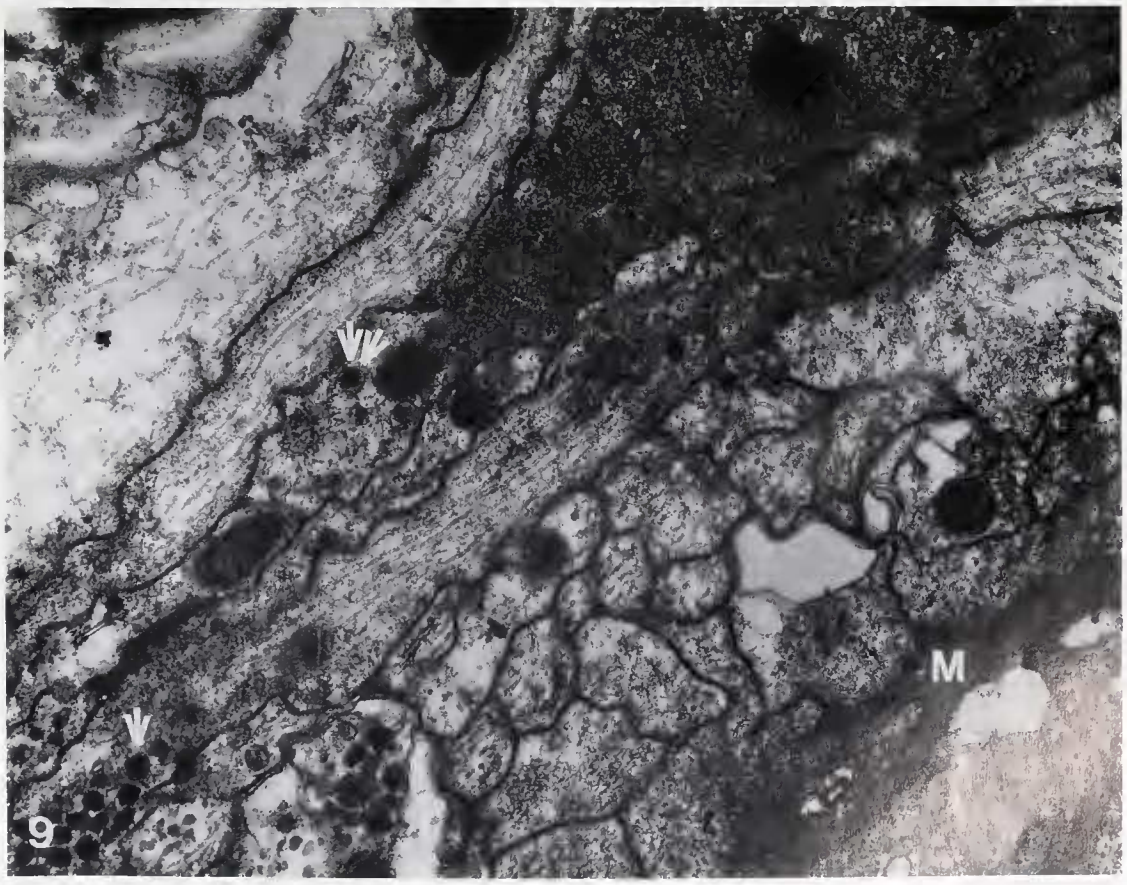


Figure 8. Dark ganglionic cell in a 72 hour planula. The perikaryon is oriented parallel to the mesoglea (M). EC, ectoderm; EN, endoderm. ×10,600.

Figure 9. Enlargement of the neurite region of the dark ganglionic cell seen in Figure 8. Both electron-dense droplets (single arrow) and dense cored vesicles (double arrows) fill the neurites of these dark cells. M, mesoglea. ×27,500.

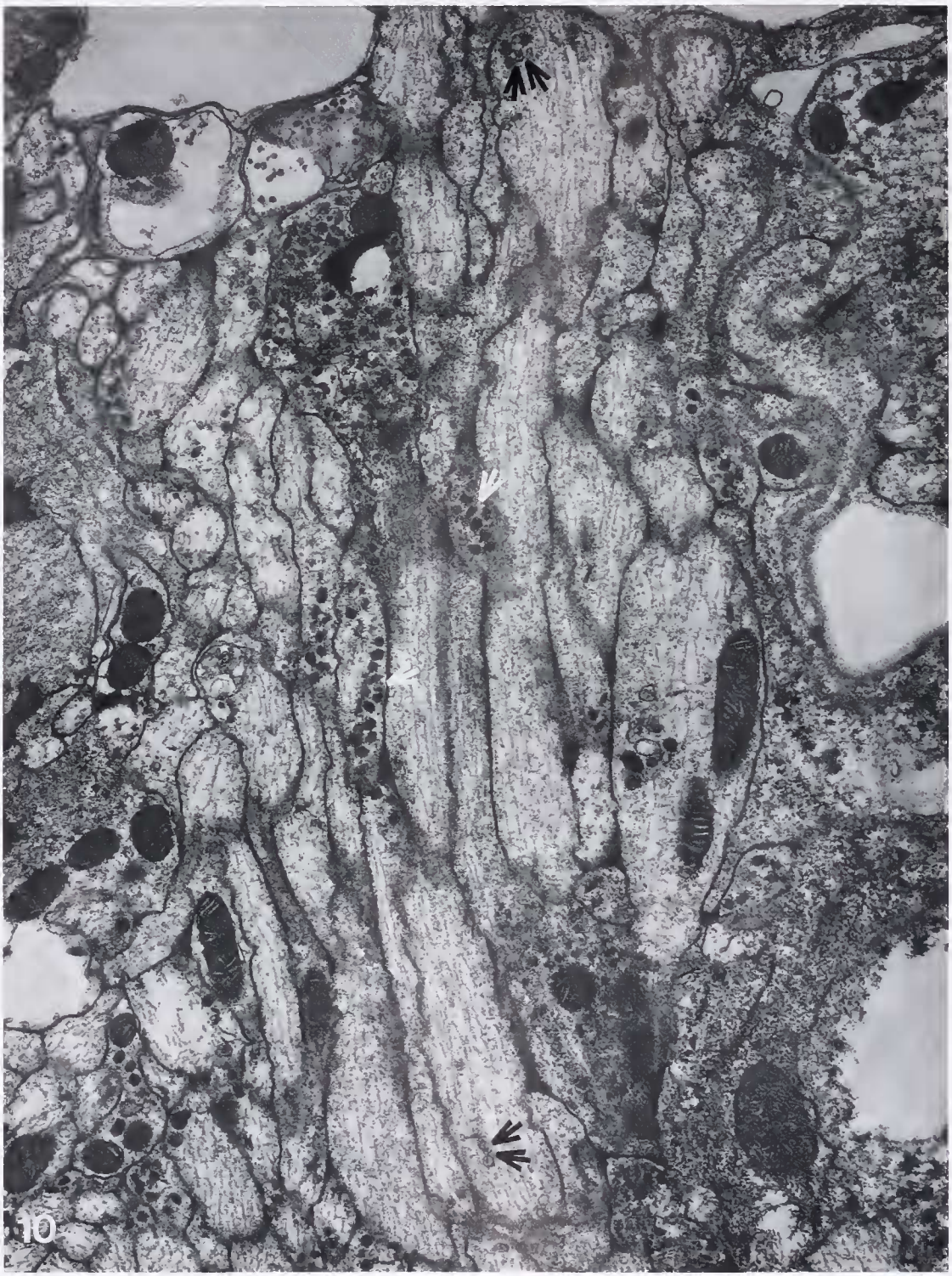
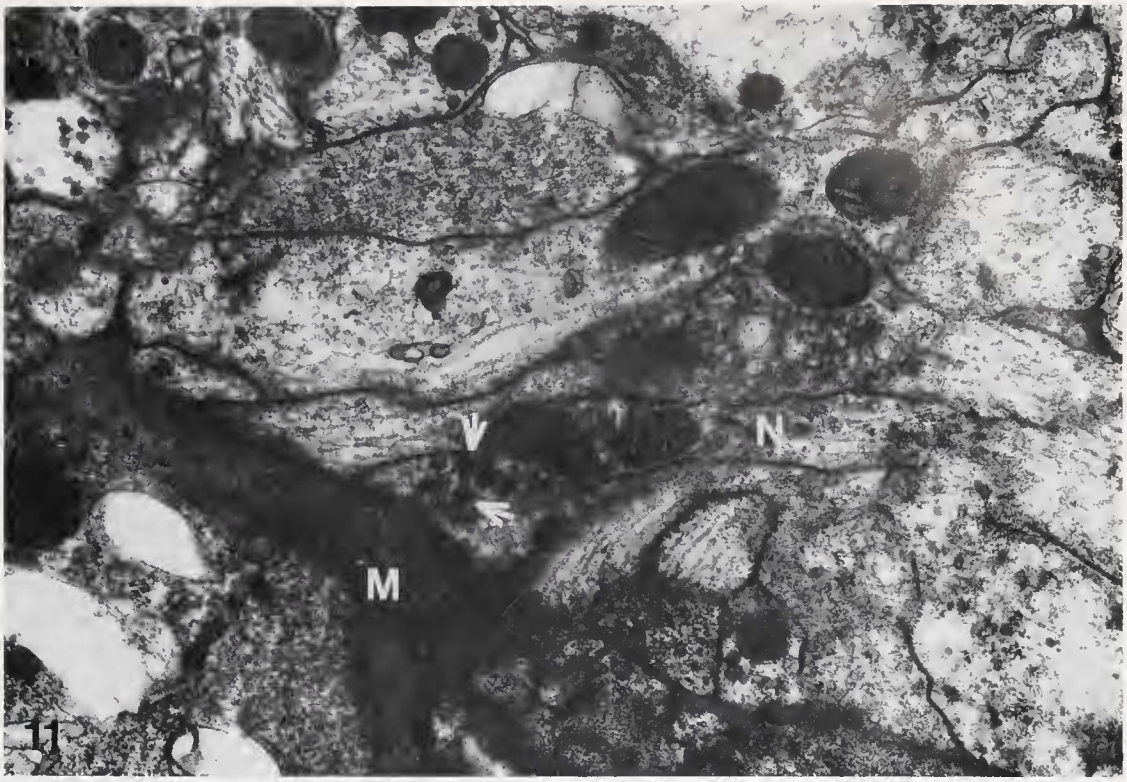


Figure 10. Well-developed nerve plexus at the base of the ectoderm of a 72 hour planula. Tracks of neurites rich in microtubules, mitochondria, electron-dense droplets (single arrow), and dense cored vesicles (double arrows) are conspicuous. These droplets and vesicles are located in clusters at certain positions along the neurites. ×18,000.



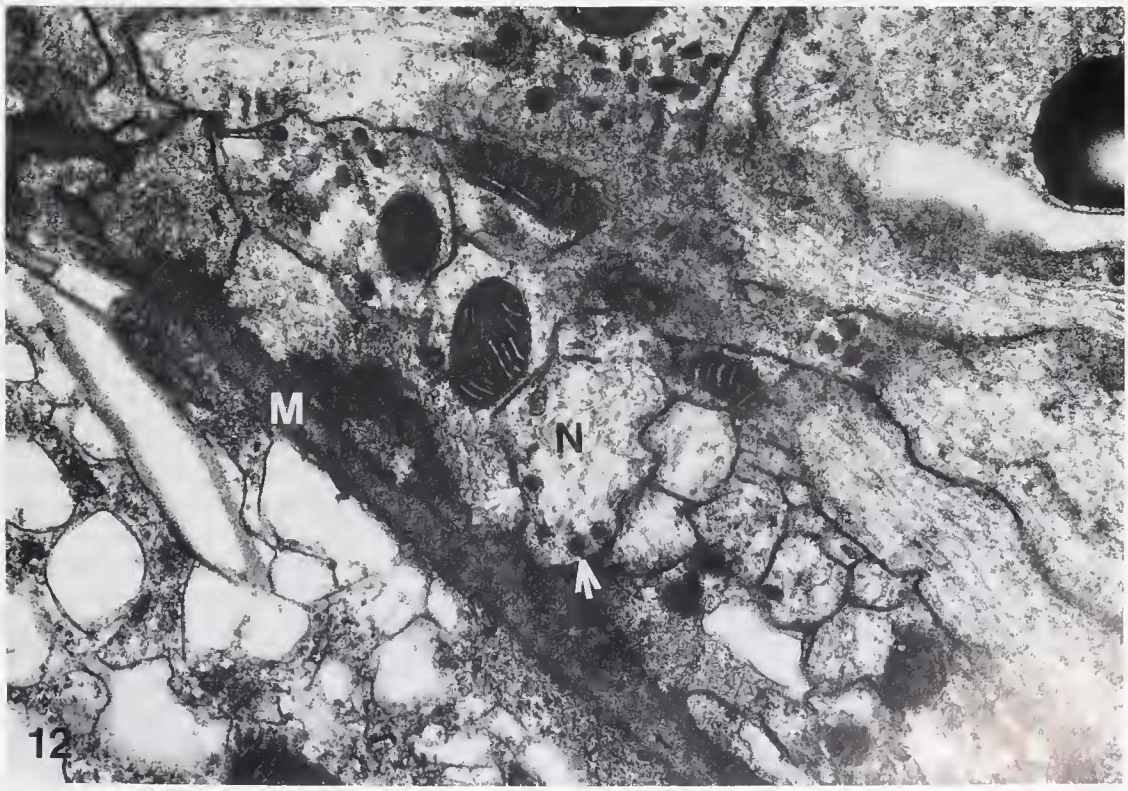


Figure 11. Neurites abutting the mesoglea (M) in a mature planula. Electron-dense droplets (arrows) of the neurites (N) are seen in close proximity to the mesoglea. ×27,500.

Figure 12. Dense cored vesicles (arrows) of neurites (N) in intimate association with the mesoglea (M). ×27,500.

neurites shortly after the appearance of the electron-dense droplets in the neurites. The fully differentiated ganglionic cell is characterized by a centrally located nucleus, a cytoplasm rich in microtubules and mitochondria, a cytoplasm containing a Golgi complex and electron-dense droplets and dense cored vesicles, and neurites extending from both sides of the cell body (Figs. 6, 7).

Two morphological types of ganglionic cells are found in the planula: a light ganglionic cell and a dark ganglionic cell (Figs. 6–9). Both types of ganglionic cells differentiate in the same manner (*i.e.*, pass through the same morphological stages of differentiation) as described above. Light ganglionic cells have an electron-lucent cytoplasm, possess fewer mitochondria than dark ganglionic cells, and their cell bodies are rounder than those of dark ganglionic cells (Fig. 7). Dark ganglionic cells possess an electron-dense cytoplasm, a small nucleus, and numerous mitochondria (Figs. 7, 8). The cell bodies of dark ganglionic cells are more oblong than those of light ganglionic cells, with their oblong axes oriented parallel to the mesoglea (Fig. 8). Light ganglionic cells comprise the majority of the ganglionic cell population and first appear at 24 hours postfertilization. A few dark ganglionic cells are present in 24 hour planulae. However, the majority of dark ganglionic cells arise later in planular development (48–96 hours postfertilization) just prior to metamorphosis. Light ganglionic cells are distributed along the whole anterior-posterior axis of the planula, whereas dark ganglionic cells are found in the head and anterior mid-sides of the planula. Both types of ganglionic cells contribute neurites to the neural plexus (Figs. 9–12).

The ectodermal neural plexus, located apical to the mesoglea, consists of transversely and longitudinally oriented processes which are present along the entire length of the planula (Figs. 10–12). The plexus of neurites is detected as early as 24 hours postfertilization and increases in size (*i.e.*, the number of neurites increase) and complexity as the planula matures. Electron-dense droplets and dense cored vesicles are abundant in the plexus and are found in clusters in specific regions of the neurites and are not evenly distributed along the entire length of the neurites (Fig. 10). Neurites extend down from the plexus and abut the mesoglea (Figs. 11, 12). In the region of neurite contact with the mesoglea electron-dense droplets and dense cored vesicles are found. The neural plexus in the head region of the planula is more extensive than the more posterior neural plexus, however there does not appear to be an accumulation of ganglionic cells in the head region.

Discussion

Hydrozoan planulae provide good embryonic systems in which to examine neural differentiation. In many planulae the entire nervous system forms within a period of a few days (Martin and Thomas, 1980; Martin *et al.* 1983). In *Halocordyle disticha* planulae, fully differentiated ganglionic cells first appear in the ectoderm as early as 24 hours postfertilization. These ganglionic cells are derivatives of endodermal interstitial cells which arise between 8–10 hours postfertilization. Ganglionic cell differentiation occurs rather quickly (at most 14–16 hours are needed for the first ganglionic cells to appear in the ectoderm once interstitial cells form); it is also easily characterized using transmission electron microscopy: appearance of a Golgi complex, mitochondria, microtubules, droplets, vesicles, and neurites. By 24 hours a neural plexus composed of neurites from both ganglionic cells and from sensory cells is prominent just above the mesoglea (Martin, unpub. obs.). This plexus increases in diameter and acquires more droplets and vesicles, and more ganglionic cells differentiate and insert their neurites into the plexus as the planula matures. Thus by 48–96 hours the nervous system of the planula (sensory cells and ganglionic cells) is quite extensive and well-organized.

Two types of ganglionic cells are morphologically identifiable at the fine-structural level in *Halocordyle disticha* planulae: a light ganglionic cell and a dark ganglionic cell. Light ganglionic cells have been observed in other hydrozoan planulae; the dark ganglionic cell has not been previously identified (Martin *et al.*, 1983). A few dark ganglionic cells are found in 24 hour planulae, however the majority of dark ganglionic cell differentiation occurs later in planular life (just prior to metamorphosis). The fact that dark ganglionic cells increase in number just prior to metamorphosis may indicate their involvement in the metamorphic process.

Martin and Thomas (1981b) proposed a neural mechanism of control of metamorphosis in hydrozoan planulae. They suggested that sensory cells of the planula perceive the necessary stimulus for attachment and metamorphosis and convey this information to ganglionic cells. In turn, ganglionic cells via either neurosecretion (chemical) and/or neurotransmission (electrical) transmit the metamorphic stimulus to the remaining larval cell types. The morphology and architecture of the planular nervous system of *Halocordyle disticha* support Martin and Thomas's (1981b) theory. Sensory cells of the planula make contact with the external environment and also feed neurites into the ganglionic plexus (Martin, unpub. obs.). Hence it is easy to visualize an integrative neural system in the ectoderm. The endoderm, however, lacks neural elements. This study demonstrates that ectodermal neurites make contact with the mesoglea. Furthermore, in the region of such contact electron-dense droplets and dense cored vesicles are found. If ganglionic cells function in neurotransmission and/or neurosecretion, then one can now visualize how a metamorphic

stimulus could also be propagated rather quickly to the endoderm. The vesicles in contact with the mesoglea could be discharged, releasing their products into the mesoglea and ultimately to the endodermal cells. Thus both ectodermal and endodermal larval cells would be able to respond rapidly to an appropriate metamorphic stimulus, even though there are no nerves in the planular endoderm.

Ganglionic cells (both dark and light) of the planula exhibit combined morphological features of interneurons and neurosecretory cells and bear some resemblance to the sensory-motor-interneurons (ganglion cells) described for adult *Hydra* (Westfall, 1973). Neurites of planular ganglionic cells make contact with neurites of adjacent ganglionic cells and with neurites of sensory cells (Martin, unpub. obs.) and hence resemble interneurons. Planular neurites also abut the mesoglea. Furthermore, planular ganglionic cells and their neurites contain numerous droplets and vesicles. Recently, Martin (1987) and Kolberg and Martin (1987) demonstrated neuropeptide-like substances and catecholamines in association with some of the ganglionic cells and their neurites. Hence, the planular ganglionic cell has combined features of an interneuron and a neurosecretory cell. Westfall and Kinnamon (1978) proposed that such multifunctional features combined into a single neuron might indicate that the neuron is a primitive stem cell from which evolved more specialized nerve cells found in higher animals. The present study suggests that the planula, the larval form of cnidarians, contains multifunctional neurons.

Acknowledgments

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